

## AMENDMENT

### **In the Specification:**

Please replace the paragraph beginning at page 1, line 9 (immediately after the title) with the following rewritten paragraph:

--The present application is a continuation of USSN 09/383,667, now allowed; which claims priority under 35 U.S.C. § 119 to provisional application numbers 60/122,767, filed March 3, 1999 and 60/098,233, filed August 28, 1998, both abandoned.--

Please replace the paragraph beginning at page 1, line 30 with the following rewritten paragraph:

--The Gla domain of FIX/FIXa contains important structural determinants for interaction with high affinity binding sites on vascular endothelial cells and platelets (Heimark et al., (1983) Biochem. Biophys. Res. Commun. 111:723-731; Ahmad et al., (1994) Biochem. 33:12048-12055; Ryan et al., (1989) J. Biol. Chem. 264:20283-20287; Toomey et al., (1992) Biochemistry 31:1806-1808; Cheung et al., (1992) J. Bio. Chem. 267:20529-20531; Rawala-Sheikh et al., (1992) Blood 79:398-405; Cheung et al., (1996) Proc. Natl. Acad. Sci. USA 93:11068-11073; Prorok et al., (1996) Int. J. Pept. Prot. Res. 48:281-285; Ahmad et al., (1998) Biochemistry 37:1671-1679). In the presence of Ca<sup>++</sup> and Mg<sup>++</sup> the FIX/FIXa Gla domain adopts different conformations. Coagulation reactions, such as FIX/FIXa-mediated activation of FX proceed with high efficiency on the surface of activated platelets (Ahmad and Walsh (1994) Trends Cardiovasc. Med., 4:271-277).--

Please replace the paragraph beginning at page 2, line 11 with the following rewritten paragraph:

--Antibodies that bind the FIX/FIXa Gla domain have been shown to inhibit FIX/FIXa function, such as cell binding (Cheung et al., (1996) supra; clotting activity (Sugo et al., (1990) Thromb. Res. 58:603-614) and FIX/FIXa activation by FXI (Sugo et al., (1990) supra; Liebman et al., (1987) J. Bio. Chem. 262:7605-7612). Rabbit and murine antibodies to FIX/FIXa have been shown to bind to the C- and N-terminal region of the Gla domain (Liebman et al., (1993) Eur. J. Biochem. 212:339-345 and Sugo et al., (1990) Thromb. Res. 58:603-614). Antibodies reactive with human FIX/IXa have been shown to inhibit the activation of FIX to FIXa and

inhibit coagulation in a FIXa dependent assay (Blackburn et al., (1997) Blood 90:Suppl. 1:424a-425a). Active site inhibited FIXa attenuates thrombosis in vivo (Wong et al., (1997) Thromb. Haemost. 77:1143-1147; Benedict et al., (1991) J. Clin. Invest. 88:1760-1765; Spanier et al., (1998) J. Thoracic Cardiovasc. Surgery 115:1179-1188).--

Please replace the paragraph beginning at page 10, line 24 with the following rewritten paragraph:

--An FIX/FIXa mediated or associated process or event, or equivalently, an activity associated with plasma FIX/FIXa, according to the present invention is any event which requires the presence of FIX/IXa. The general mechanism of blood clot formation is reviewed by Ganong, in Review of Medical Physiology, 13th ed., Lange, Los Altos, CA, pp411-414 (1987); Bach (1988) CRC Crit. Rev. Biochem. 23(4):339-368 and Davie et al., (1991) Biochemistry 30:10363; and the rate of FIX in Limentani et al., (1994) Hemostasis and Thrombosis Basic Principles and Clinical Practice, Third Edition, Coleman et al. Eds., Lippincott Company, Philadelphia. Coagulation requires the confluence of two processes, the production of thrombin which induces platelet aggregation and the formation of fibrin which renders the platelet plug stable. The process comprises several stages each requiring the presence of discrete proenzymes and procofactors. The process ends in fibrin crosslinking and thrombus formation. Fibrinogen is converted to fibrin by the action of thrombin. Thrombin, in turn, is formed by the proteolytic cleavage of prothrombin. This proteolysis is effected by FXa which binds to the surface of activated platelets and in the presence of FVa and calcium, cleaves prothrombin. TF-FVIIa is required for the proteolytic activation of FX by the extrinsic pathway of coagulation. FIX is activated by two different enzymes, FXIa (Fujikawa et al., (1974) Biochemistry, 13:4508-4516; Di Scipio et al., (1978) J. Clin. Invest., 61:1528-1538; Østerud et al., (1978) J. Biol. Chem. 253:5946-5951) and the tissue factor:factor VIIa (TF:FVIIa) complex (Østerud and Rapaport (1977) Proc. Natl. Acad. Sci. USA 74:5260-5264). The formed FIXa in complex with its cofactor FVIIIa assembles into the intrinsic Xase complex on cell surfaces such as platelets and endothelial cells, and converts substrate FX into FXa (Mann et al., (1992) Semin. Hematol. 29:213-226). Thrombin generated by FXa enzymatic activity, cleaves fibrinogen leading to fibrin formation and also activates platelets resulting in platelet aggregation. Therefore, a

process mediated by or associated with FIX/IXa, or an activity associated with FIXa includes any step in the coagulation cascade from the introduction of FIX in the extrinsic or intrinsic pathway to the formation of a fibrin platelet clot and which initially involves the presence FIX/IXa. FIX/FIXa mediated or associated process, or FIXa activity, can be conveniently measured employing standard assays such as those described herein.--

Please replace the paragraph beginning at page 21, line 25 with the following rewritten paragraph:

--Further provided herein are an antibody or antibody fragment comprising any of the heavy chain CDR sequences as described above, and further comprising a light chain CDR amino acid sequence comprising the amino acid sequence of a light chain CDR amino acid sequence of Figure 2. By way of example, in one embodiment, the invention provides a single chain antibody fragment wherein any heavy chain comprising a CDR1 a CDR2 and a CDR3, and light chain ( $\lambda$ c) comprising a  $\lambda$ c-CDR1, a  $\lambda$ c-CDR2 and a  $\lambda$ c-CDR3 are contained in a single chain polypeptide species. By way of example and not limitation, the single chain antibody fragment is, in a particular embodiment, a scFv species comprising the heavy chain joined to the light chain by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the heavy chain joined to the light chain by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e., a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.--

Please replace the paragraph beginning at page 26, line 26 with the following rewritten paragraph:

--Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table A under the heading of "preferred substitutions". If such substitutions result in a change in

biological activity, then more substantial changes, denominated "exemplary substitutions" in Table A, or as further described below in reference to amino acid classes, may be introduced and the products screened.--

Please replace Table A beginning at page 27, line 3 with the following rewritten Table A:

Table A

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; leu; ile	val
Arg (R)	Lys; gln; asn	lys
Asn (N)	Gln; his; asp, lys; arg	gln
Asp (D)	Glu; asn	glu
Cys (C)	Ser; ala	ser
Gln (Q)	Asn; glu	asn
Glu (E)	Asp; gln	asp
Gly (G)	Ala	ala
His (H)	Asn; gln; lys; arg	arg
Ile (I)	Leu; val; met; ala; phe; norleucine	leu
Leu (L)	Norleucine; ile; val; met; ala; phe	ile
Lys (K)	Arg; gln; asn	arg
Met (M)	Leu; phe; ile	leu
Phe (F)	Leu; val; ile; ala; tyr	tyr
Pro (P)	Ala	ala
Ser (S)	Thr	thr
Thr (T)	Ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Please replace the paragraph beginning at page 47, line 12 with the following rewritten paragraph:

--Antibody variants in which residues have been deleted, inserted, or substituted are recovered in the same fashion, taking account of any substantial changes in properties occasioned by the variation. For example, preparation of an antibody fusion with another protein or polypeptide, e.g., a bacterial or viral antigen, facilitates purification; an immunoaffinity column containing antibody to the antigen can be used to adsorb the fusion polypeptide. Immunoaffinity columns such as a rabbit polyclonal anti-antibody column can be employed to absorb the antibody variant by binding it to at least one remaining immune epitope. Alternatively, the antibody may be purified by affinity chromatography using a purified FIX Gla

domain-IgG coupled to a (preferably) immobilized resin such as AFFI-Gel 10 (Bio-Rad, Richmond, CA) or the like, by means well known in the art. A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. One skilled in the art will appreciate that purification methods suitable for the native antibody may require modification to account for changes in the character of the antibody or its variants upon expression in recombinant cell culture.--

Please replace the paragraph beginning at page 65, line 20 with the following rewritten paragraph:

--Cross-species reactivity of 10C12 F(ab')<sub>2</sub> - The amino acid sequences of FIX-Gla domains of different animal species are much conserved (Fig. 1A), suggesting that an antibody that binds to human FIX-Gla may also recognize plasma FIX/IXa of various animals. The potency of 10C12 F(ab')<sub>2</sub> to inhibit the APTT in plasma from different species was therefore examined. As shown in Figure 9B, 10C12 F(ab')<sub>2</sub> most potently prolonged the APTT in dog and to a lesser extent than in rat and rabbit plasma. The specificity of the antibody effect towards FIX/IXa was evidenced by the absence of any effect on the PT in homologous plasma.--